H36.D2.B7 ANTI-TISSUE FACTOR LIGHT CHAIN VARIABLE REGION

GACATTCAGATGACCCAGTCTCCTGCCTCCCAGTCTGCATCTCTGGGAGAAAGTGTCACCATCACATGC PASQSA'S LGESVTI ഗ თ ⊢ **Σ** Ø CTGGCAAGTCAGACCATTGATACATGGTTAGCATGGTATCAGCAGAAACCAGGGAAATCTCCTCAGCTC D T W L A W Y Q Q K P G K S P Ø တ

CTGATTTATGCTGCCACCAACTTGGCAGATGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGCACA ഗ A D G V P S R F S G S G S Z

<u> AAATTTTCTTTCAAGATCAGCAGCCTACAGGCTGAAGATTTTGTAAATTATT TACTGT<u>CAACAAGTTTA</u>C</u> SSILQAEDFVNY

AGTTCTCCATTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA S S P F T F G A G T K L E L K

FIG. 1A

H36.D2.B7 ANTI-TISSUE FACTOR HEAVY CHAIN VARIABLE REGION

GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGCAGGTATCCTGCAAG Q L Q Q S G P E L V K P G A S V Q V S

ACTTCTGGTTACTCATTCACTGACTACAACGTGTACTGGGTGAGGCCAGAGCCATGGAAAGAGGCCTTGAG SGYSF<u>TDYNVY</u>WVRQSHGKSL

TGGATTGGA<u>TATATTGATCCTTACAATGGTATTACTATCTACGACCAGAACTTCAAGGGC</u>AAGGCCACA W | G Y | D P Y N G | T | Y D Q N F K G K A TTGACTGTTGACAAGTCTTCCACCACAGCCTTCATGCATCTCAACAGCCTGACGACTCTGCA

GTTTATTTCTGTGCAAGAGATGTGACTACGGCCCTTGACTTCTGGGGCCCAAGGCCACCACTCTCACAGTC
V Y F C A R D V T T A L D F W G Q G T T L T V F W G V C

TCCTCA

FIG. 1B

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ANTIBODY	APPARENT K ₁ , M ⁻¹	APPARENT K ₁ , M
BY ELISA		
D2	5.2 x 10 ⁹	1.9 x 10 ⁻¹⁰
147	6.5 x 10 ⁹	1.5 x 10 ⁻¹⁰
K73	9.8 x 10 ⁹	1.0×10^{-10}
K80	2.3×10^9	4.3×10^{-10}
L102	2.5 x 10 ⁹	4.0×10^{-10}
L133	1.7×10^9	5.9 x 10 ⁻¹⁰
BY BIACore		
H36	3.1 x 10 ¹⁰	3.2 x 10 ⁻¹¹
143	2.3 x 10 ⁹	4.3 x 10 ⁻¹⁰
147	3.2×10^9	3.1 x 10 ⁻¹⁰
L133	4.6×10^9	2.2×10^{-10}
M107	1.1 x 10 ⁹	9.1 x 10 ⁻¹⁰

FIG. 2

ANTIBODY NAME	% INHIBITION ANTIBODY PREINCUBATED WITH TF/VIIa
D1	0
D1B	1
H31	4
H36	95
143	1
J131	7
K80	0
K82	0
K87	1
L97B	7
. L101	0
L102	0
L105	0
L133	0
M5	1
M107	34

ANTIBODY NAME	% INHIBITION TF PREINCUBATED WITH ANTIBODY PRIOR TO ADDITION OF VIIa	% INHIBITION TF PREINCUBATED WITH VIIa PRIOR TO ADDITION OF ANTIBODY
	7.DDTTTOTY OF VIIG	
D1	15	nd
D1B	48	12.7
H31	64	21
H36	0	0
143	68	55
J131	38	11
K80	12	nd
K82	0	nd
K87	0	nd
L96	0	nd
L101	38	11
L102	14	nd
L105	4	nd
L133	13	nd
M5	0	nd
M107	0	nd

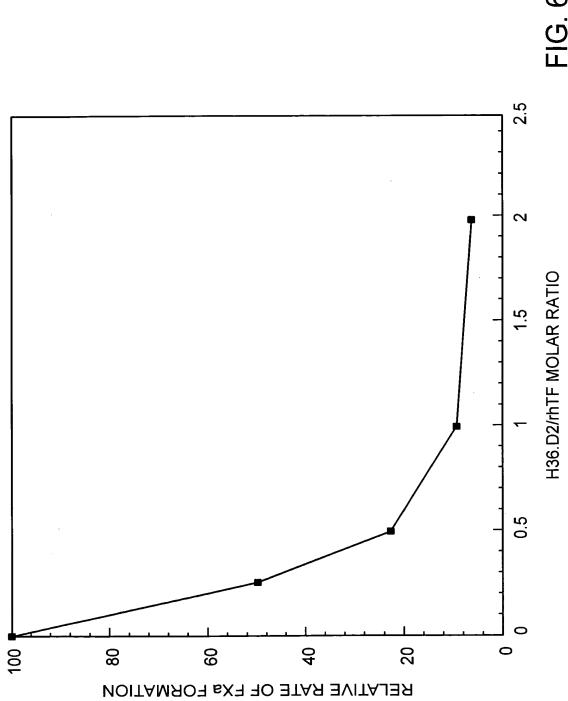
FIG. 4

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[rhTF], nM	[H36.D2], nM	H36.D2/rhTF MOLAR RATIO	CLOTTING TIME (SECONDS)	% INHIBITION OF rhTF FUNCTION
0.0048	0	0	102.3	0
	1.61	335.4	114.3	31.3
	3.23	670.8	121.3	45.8
0.023	0	0	77.6	0
	1.61	70.0	85.3	52.2
	3.23	140.0	91.1	65.2
	6.45	280.4	99.6	73.9
0.092	0	0	49.3	0
	3.23	35.1	65.8	65.2
	6.45	70.1	88.5	90.2
	12.90	140.2	113.3	95.7
0.46	0	0	32.6	0
	6.45	14.0	52.7	82.4
	12.90	28.0	80.2	96.7
	32.30	70.2	117.9	99.3
2.30	0	0	23.9	0
	16.10	7.0	47.1	94.4
	32.30	14.0	95.2	99.7
	64.50	28.0	115.3	99.9
11.52	0	0	22.2	0
	16.10	1.4	30.2	93.4
	32.30	2.8	46.0	98.8
	64.50	5.6	87.6	99.9
	161.30	14.0	114.0	100.0

FIG. 5





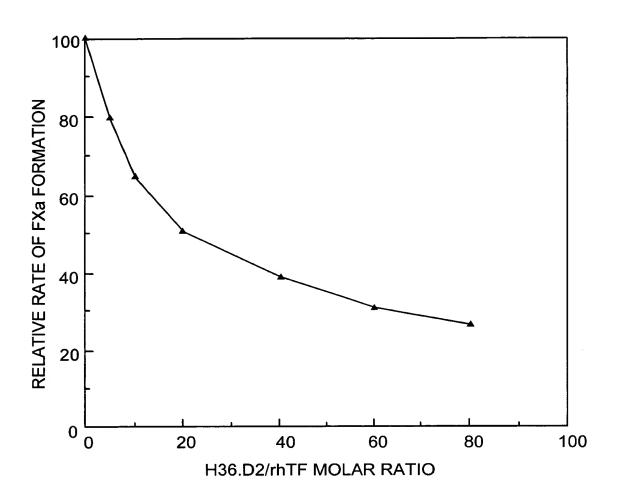


FIG. 6B

% INHIBITION FX AND H36.D2 ARE ADDED SIMULTANEOUSLY TO CELLS (TF/FVII)	0	ри	pu	pu	92	78	92
% INHIBITION CELLS (TF/FVII) AND H36.D2 PREINCUBATED PRIOR TO FX ADDITION	0	88	92	26	pu	þu	pu
H36.D2 CONCENTRATION (ng)	0	20	100	200	800	1600	3200

FIG. 7

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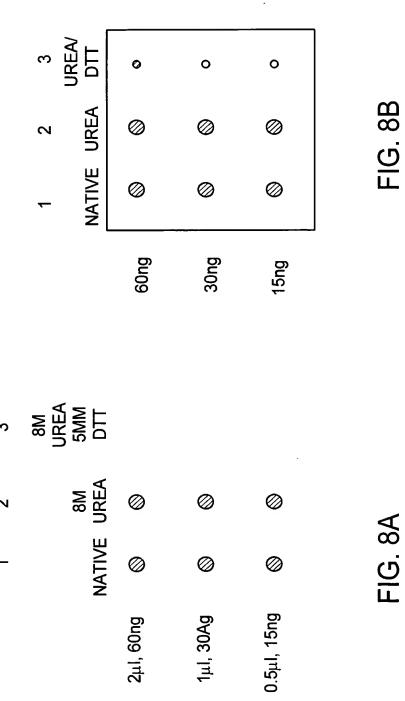


FIG. 8B

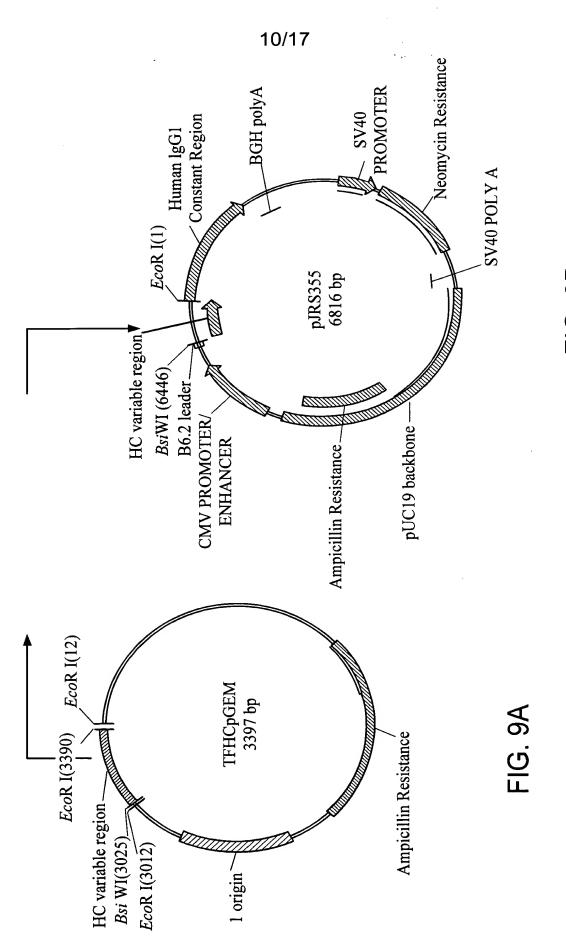


FIG. 9B

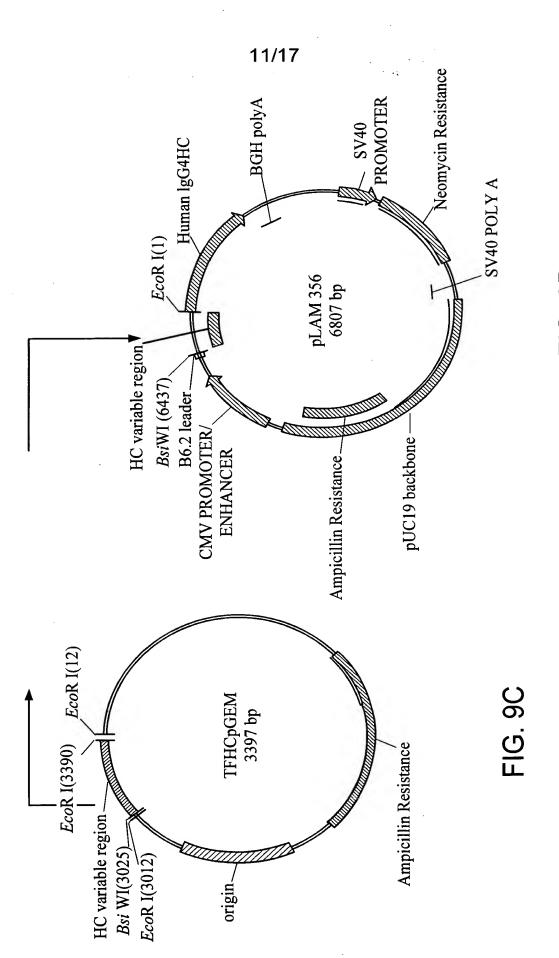


FIG. 9D

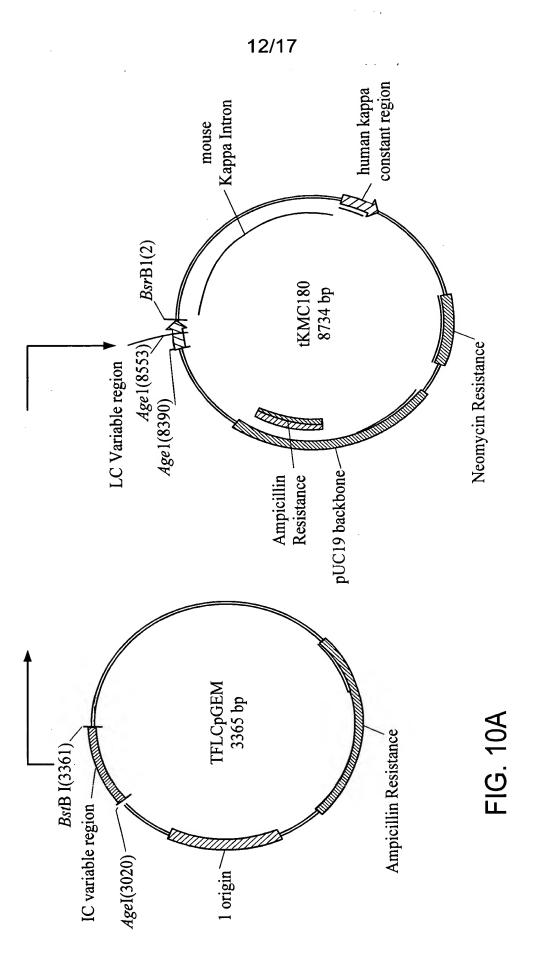


FIG. 10B

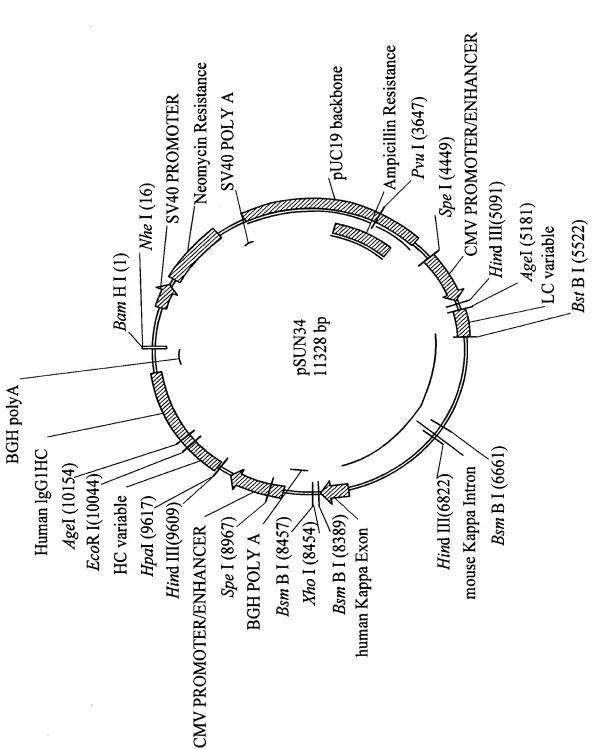


FIG. 11

Humanization of anti-Tissue Factor Antibody cH36

Sequences of Partially and Fully Humanized Light Chain (LC) Variable Regions Light Chain (LC) FR Sequences

Names		CH36-LC	IC-03	LC-04	IC-05	TC-06	IC-07	IC-08	IC-09	LC-10	LC-11	LC-12
FR4 (10 AA)	98 107	FGAGTKLELK	FGAGTKLETK	FGAGTKLEIK	FGOGTKLEIK	FGOGTKLEIK	FGOGTKLEIK	FGOGTKLETK	FGOGTKLETK	FGOGTKLETK	FGOGTKLETK	FGOGTKLEIK
FR3 (32 AA)	47 57 60 70 80 86	DIQMTQSPASQSASLGESVTITC WYQQKPGKSPQLIY GVPSRFSGSGSGTKFSFKISSLQAEDFVNYYC	GVPSRFSGSGSGTKFSFKISSLQAEDFVNYYC	GVPS FSGSGSGTKFSFKISSLQAEDFVNYYC	GVPSRFSGSGSGTKFSFKISSLQAEDFVNYYC	GVPSRFSGSGSGTKFSFKISSLQAEDFVNYYC	GVPSRFSGSGSGTDFSFTTSSLOPEDFVNYYC		GVPSRFSGSGSGTDFSFTISSLOPEDFATYYC		GVPSRFSGSGSGTKFSFITSSLOPEDFPNYYC	
FR2 (14 AA)	35 47	TC WYQQKPGKSPQLIY	TC WYQQKPGKSPQLIY	TC WYLIOKPGKSPOLIY	TC WYLOKPGKSPOLIY	TC WYLOKPGKSPOLIY	TC WYLIOKPGKSPOLIY	TC WYLQKPGKSPQLIY	TC WYLOKPGKSPOLIY	TC WYLLOKPGKSPOLIY	TC WYLOKPGKSPOLIY	TC WYLOKPGGSPOLIY
FR1 (23 AA)	1 10 20	DIQMTQSPASQSASLGESVTI	DIQMTQSPASQSASLGESVTITC WYQQKPGKSPQLIY	DIOMIQSPASQSASLGESVIIIC WYJOKPGKSPOLLY	DIOMIOSPASISASWGDRVIIIC WYLOKPGKSPOLIY	DIOMIOSPASOSASLGESVIIIC WYLOKPGKSPOLIY	DIOMTOSPASOSASLGESVTITC: WYLOKPGKSPOLIY	DIOMTOSPASOSASLGESVTITC WYLOKPGKSPOLIY	DIOMIOSPASIISASWGDRVIIIC WYDOKPGKSPOLIY	DIOMIOSPASIISASWGDRVIIIC WYLOKPGKSPOLIY	DIOMIQSPASISASVGDRVIIIC WYLOKPGKSPQLIY	DIOMTOSPASIJSASIVGIDRIVTITC WYLJOKPGOSPOLIY

FIG. 12A

of cH36	CDR2 (7 AA)	90 20	AATNIAD	FIG. 12C
Light Chain CDR Sequnces	CDR1 (11 AA)	24 34	LASQTIDIWLA	FIG. 12B

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CDR3 (9 AA)

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15/17

Sequences of Partially and Fully Humanized Heavy Chain (LC) Variable Regions

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FR1 (30 AA)	FR2 (14 AA)	FR3 (32 AA) FR4 (11 AA)	Names
1 10 20 29	36 44	85 95 1	
EIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSHGKSLEWIG	KATLIVDKSSTIAFMHLNSLISDDSAVYFCAR WGQGTTLIVSS	CH36-HC
OTOLOOSGPELVKPGASVQVSCKTSGYSFT	WVRQSHGKSLEWIG	KATLIVDKSSTIAFMHLNSLISDDSAVYFCAR WGQGTIÑIVSS	HC-01
QIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSPGKGLEWIG	KATLIVDKSSTTAFMHLNSLTSDDSAVYFCAR WGQGTTVTVSS	HC-02
QIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSPGKGLEWIG	KATLIVDKSSTTAFMHINSLRSEDIRVYFCAR WGQGTTVIVSS	HC-03
OIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSPGKGLEWIG	KATLIVDKSSTTAFMELESLASEDIAVYFCAR WGQGTTMTVSS	HC-04
QIQLQQSGPELVKPGASVQVSCKTSGYSFT	WVRQSPGKGLEWIG	KATLIVDKSTSTANDLISSLASEDTAVYFCAR WGQGTTVTVSS	HC-05
OMOLOOSGGELVKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLIVDKSITSITAKWELISISLRISEDIAVYFCAR WGQGITIKITVSS	HC-06
CIOIWOSGGELVKPGASVRIVSCKASGYSFT	WVRQSPGKGLEWIG	KATLIVDKSTSTAMMELISSLESEDTAVYFCAR WGQGTTMTVSS	HC-07
QIQIWQSGGEVKKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLIVDKSISIRAMELISSLESEDIAVYFCAR WGQGITIVIVSS	HC-08
QIQIWOSGGEWKKPGASWRWSCKASGYSFT	WVRQSPGKGLEWIG	KATLIVDKSIISIAMELISSLRSEPIAVYFCAR WGQGIIMIVSS	HC-08R1
OIOIVOSGPEVKKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTAMMELISSLRSEDTAVYFCAR WGQGTTMTVSS	HC-11
CIOUVOSGPELKKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLIVDKSTSTAMELSSLRSEDTAVYFCAR WGQGTTWTVSS	HC-12
OTOLWOSGPELVKPGASVRVSCKASGYSFT	WVRQSPGKGLEWIG	KATLTVDKSTSTANNELSSLKSEDTAVYFCAR WGQGTTVTVSS	HC-09
OI OLIVOSGPEVIVKPGASVRVSCKASGYSFT	WVRQS <u>PGKG</u> LEWIG	KATLTVDKS <u>TSTAMPLESSLESSEDTAV</u> YFCAR WGOGTTWTVSS	HC-10

-1G. 13A

Heavy Chain CDR Sequnces

Names	HC-08	
CDR3 (8AA)	DVITALDE PIC 120	
CDR2 (17 AA)	YIDPYNGITIYDONEKG TIC 130	
CDR1 (5 AA)		
;	H K	ロペナ <u>リ</u> 山

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hoat (1gG1) constant regions sequences

SEQUENCES OF LC CONSTANT:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH

KVYACEVTHQGLSSPVTKSFNRGEC

FIG. 14A

SEQUENCES OF HC CONSTANT:

EFASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

FIG. 14B

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FIG. 15A

HKVYACEVTHQGLSSPVTKSFNRGEC

SEQUENCES OF LC CONSTANT:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK

hFAT (IgG1) CONSTANT REGION SEQUENCES

SEQUENCES OF HC CONSTANT:

TCLVKGFYPSDDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGK EFASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTY EVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSL TCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGV

FIG. 15B